<u>AMENDMENTS TO THE CLAIMS</u>

1.   (Currently amended) A method for selecting from a plurality of different siRNAs one or more siRNAs for silencing a target gene in an organism, each [[of]] <u>different siRNA in said plurality of different siRNAs</u> targeting a different target sequence in a transcript of said target gene, said method comprising

<u>(a) calculating a score for a corresponding targeted sequence motif in said transcript, for each said different siRNA in said plurality of different siRNAs, wherein said score is calculated using a position-specific score matrix (PSSM), wherein each said sequence motif is a nucleotide sequence of L nucleotides, L being an integer, and wherein said PSSM is $\{\log(e_{ij}/p_{ij})\}$, where e_{ij} is the weight of nucleotide i at position j, p_{ij} is the weight of nucleotide i at position j in a random sequence, and $i = G$ or C, A, $U(T)$, $j = 1, ..., L$, wherein each of said targeted sequence motifs comprises at least a portion of the target sequence of the corresponding siRNA and/or a second sequence in a sequence region flanking said target sequence;</u>

[[(a)]] <u>(b)</u> ranking said plurality of different siRNAs according to ~~positional base compositions of a corresponding targeted sequence motifs in said transcript, wherein each said targeted sequence motif comprises at least a portion of the target sequence of the corresponding siRNA and/or a second sequence in a sequence region flanking said target sequence~~ <u>said scores</u>; and

<u>(c)</u> selecting one or more siRNAs from said ranked siRNAs<u>, wherein at least one of steps (a), (b) or (c) is performed by a suitably programmed computer</u>.

2.   (Original) The method of claim 1, wherein each said sequence motif comprises said target sequence of said targeting siRNA.

3.   (Canceled)

ROSA\34293AM1.DOC

4. (Currently amended) The method of claim [[3]] 1, wherein each said sequence motif is a nucleotide sequence of L nucleotides, L being an integer, and wherein said position-specific score matrix is $\{\log(e_{ij}/p_{ij})\}$, where e_{ij} is the weight of nucleotide i at position j, p_{ij} is the weight of nucleotide i at position j in a random sequence, and $i = G, C, A, U(T), j = 1, ..., L$.

5. (Canceled)

6. (Currently amended) The method of claim [[5]] 1, wherein said score for each said siRNA is calculated according to equation

$$Score = \sum_{t=1}^{L} \ln\left(e_t / p_t\right)$$

wherein said e_t and p_t are respectively weights of the nucleotide at position t in said sequence motif as determined according to said position-specific score matrix and in a random sequence.

7. (Original) The method of claim 6, wherein each said sequence motif comprises said target sequence of said targeting siRNA and at least one flanking sequence.

8. (Original) The method of claim 7, wherein each said sequence motif comprises said target sequence of said targeting siRNA and a 5' flanking sequence and a 3' flanking sequence.

9. (Original) The method of claim 8, wherein said 5' flanking sequence and said 3' flanking sequence are each a sequence of D nucleotides, D being an integer.

10. (Original) The method of claim 9, wherein each said target sequence is a sequence of 19 nucleotides, and each said 5' flanking sequence and 3' flanking sequence are a sequence of 10 nucleotides.

11.　(Original) The method of claim 8, wherein each said target sequence is a sequence of 19 nucleotides, and each said 5' flanking sequence and 3' flanking sequence are a sequence of 50 nucleotides.

12–20. (Canceled)

21.　(Original) The method of claim 1, further comprising a step of selecting one or more siRNAs based on silencing specificity, said step of selecting based on silencing specificity comprising, (i) for each of said plurality of siRNAs, predicting off-target genes of said siRNA from among a plurality of genes, wherein said off-target genes are genes other than said target gene and are directly silenced by said siRNA; (ii) ranking said plurality of siRNAs according to the number of off-target genes; and (iii) selecting one or more siRNAs for which said number of off-target genes is below a given threshold.

22.　(Original) The method of claim 21, wherein said predicting comprises (i1) evaluating sequence of each of said plurality of genes based on a predetermined siRNA sequence match pattern; and (i2) predicting said gene as an off-target gene if said gene comprise a sequence that matches said siRNA based on said sequence match pattern.

23.　(Currently amended) The method of claim 22, wherein said step of evaluating comprises identifying an alignment of said siRNA to a sequence in a gene by a low stringency [[FastA]] FASTA alignment.

24.　(Original) The method of claim 23, wherein each said siRNA has L nucleotides in its duplex region, and wherein said match pattern is represented by a position match position-specific score matrix (pmPSSM), said position match position-specific score matrix

ROSA\34293AM1.DOC

consisting of weights of different positions in an siRNA to match transcript sequence positions in an off-target transcript $\{P_j\}$, where $j = 1, ..., L$, P_j is the weight of a match at position j.

25. (Original) The method of claim 24, wherein said step (i1) comprises calculating a position match score pmScore according to equation

$$pmScore = \sum_{i=1}^{L} \ln\left(E_i / 0.25\right)$$

where $E_i = P_i$ if position i is a match and $E_i = (1 - P_i)/3$ if position i is a mismatch; and said step (i2) comprises predicting said gene as an off-target gene if said position match score is greater than a given threshold.

26. (Original) The method of claim 25, wherein L is 19, and wherein said pmPSSM is given by Table I.

27. (Original) The method of claim 26, wherein said plurality of genes comprises all known unique genes of said organism other than said target gene.

28. (Original) The method of claim 10, wherein said position-specific score matrix (PSSM) is obtained by a method comprising

(aa) identifying a plurality of N siRNAs consisting of siRNAs having 19-nucleotide duplex region and having a silencing efficacy above a chosen threshold;

(bb) identifying for each said siRNA a functional sequence motif, said functional sequence motif comprising a 19-nucleotide target sequence of said siRNA and a 10-nucleotide 5' flanking sequence and a 10-nucleotide 3' flanking sequence;

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS^PLLC
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

-6-

ROSA\34293AM1 DOC

(cc)   calculating a frequency matrix $\{f_{ij}\}$, where $i = G,\ C,\ A,\ U(T);\ j = 1,\ 2,$ ..., L, and where f_{ij} is the frequency of the ith nucleotide at the jth position, based on said siRNAs functional sequence motifs according to equation

$$f_{ij} = \sum_{k=1}^{N} \delta_{ik}(j),$$

where $\delta_{ik}(j) = \begin{cases} 1, \text{if } k = i \\ 0, \text{if } k \neq i \end{cases}$, and

(dd)   determining said PSSM by calculating e_{ij} according to equation

$$e_{ij} = \frac{f_{ij}}{N}.$$

29.   (Original) The method of claim 28, wherein said plurality of N siRNAs target a plurality of different genes having different transcript abundances in a cell.

30.   (Original) The method of claim 29, wherein said step (b) is carried out by selecting one or more siRNAs having the highest scores.

31.   (Original) The method of claim 29, wherein said step (b) is carried out by selecting one or more siRNAs having a score closest to a predetermined value, wherein said predetermined value is the score value corresponding to the maximum median silencing efficacy of a plurality of siRNA sequence motifs.

32.   (Original) The method of claim 31, wherein said plurality of siRNA sequence motifs are sequence motifs in transcript having abundance level of less than about 3–5 copies per cell.

ROSA\34293AM1.DOC

33.   (Original) The method of claim 29, wherein said step (b) is carried out by selecting one or more siRNAs having a score within a predetermined range, wherein said predetermined range is a score range corresponding to a plurality of siRNAs sequence motifs having a given level of silencing efficacy.

34.   (Previously presented) The method of claim 33, wherein said silencing efficacy is above 50% at an siRNA dose of about 100nM.

35.   (Original) The method of claim 34, wherein said plurality of siRNA sequence motifs are sequence motifs in transcript having abundance level of less than about 3–5 copies per cell.

36.   (Canceled)

37.   (Currently amended) The method of claim [[5]] $\underline{1}$, wherein said position-specific score matrix (PSSM) is obtained by a method comprising

(aa)   initializing said PSSM with random weights;

(bb)   selecting randomly a weight w_{ij} obtained in (aa);

(cc)   changing the value of said selected weight to generate a test psPSSM comprising said selected weight having said changed value;

(dd)   calculating a score for each of a plurality of siRNAs functional sequence motifs using said test PSSM according to equation

$$Score = \sum_{k=1}^{L} \ln\left(w_k / p_k\right)$$

ROSA\34293AM1.DOC

wherein said w_k and p_k are respectively weights of a nucleotide at position k in said functional sequence motif and in a random sequence;

   (ee) calculating correlation of said score and a metric of a characteristic of an siRNA among said plurality of siRNAs functional sequence motifs;

   (ff) repeating steps (cc)–(ee) for a plurality of different values of said selected weight in a given range and retain the value that corresponds to the best correlation for said selected weight; and

   (gg) repeating steps (bb)–(ff) for a chosen number of times; thereby determining said PSSM.

  38. (Original) The method of claim 37, further comprising selecting said plurality of siRNA functional sequence motifs by a method comprising

   (i) identifying a plurality of siRNAs consisting of siRNAs having different values in said metric;

   (ii) identifying a plurality of siRNA functional sequence motifs each corresponding to an siRNA in said plurality of siRNAs.

  39. (Original) The method of claim 38, wherein said characteristic is silencing efficacy.

  40. (Original) The method of claim 39, wherein said plurality of N siRNAs target a plurality of different genes having different transcript abundances in a cell.

  41. (Original) The method of claim 40, wherein said step (b) is carried out by selecting one or more siRNAs having the highest scores.

ROSA\34293AM1.DOC

42.   (Original) The method of claim 40, wherein said step (b) is carried out by selecting one or more siRNAs having a score closest to a predetermined value, wherein said predetermined value is the score value corresponding to the maximum median silencing efficacy of a plurality of siRNA sequence motifs.

43.   (Original) The method of claim 42, wherein said plurality of siRNA sequence motifs are sequence motifs in transcript having abundance level of less than about 3–5 copies per cell.

44.   (Original) The method of claim 40, wherein said step (bb) is carried out by selecting one or more siRNAs having a score within a predetermined range, wherein said predetermined range is a score range corresponding to a plurality of siRNAs sequence motifs having a given level of silencing efficacy.

45.   (Previously presented) The method of claim 44, wherein said silencing efficacy is above 50% at an siRNA dose of about 100nM.

46.   (Original) The method of claim 45, wherein said plurality of siRNA sequence motifs are sequence motifs in transcript having abundance level of less than about 3–5 copies per cell.

47.   (Canceled)

48.   (Original) The method of claim 37, wherein said position-specific score matrix (PSSM) comprises w_k, $k = 1, ..., L$, w_k being a difference in probability of finding nucleotide G or C at sequence position k between a first type of siRNA and a second type of siRNA, and wherein said score for each said strand is calculated according to equation

ROSA\34293AM1.DOC

$$Score = \sum_{k=1}^{L} w_k .$$

49. (Original) The method of claim 48, wherein said first type of siRNA consists of one or more siRNAs having silencing efficacy no less than a first threshold and said second type of siRNA consists of one or more siRNAs having silencing efficacy less than a second threshold.

50. (Original) The method of claim 49, wherein said difference in probability is described by a sum of Gaussian curves, each of said Gaussian curves representing the difference in probability of finding a G or C at a different sequence position .

51. (Currently amended) The method of claim 50, wherein said first and second ~~threshold~~ thresholds are both 75% at an siRNA dose of 100nM.

52–113. (Canceled)

ROSA\34293AMI.DOC